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1: FEBS Lett. 1997 Jun 9;409(2):312-6.

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### **The nodulation gene *nolK* of *Azorhizobium caulinodans* is involved in the formation of GDP-fucose from GDP-mannose.**

**Mergaert P, Van Montagu M, Holsters M.**

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Department of Genetics, Flanders Interuniversity Institute for Biotechnology (VIB), Universiteit Gent, Belgium.

The *nolK* gene of *Azorhizobium caulinodans* is essential for the incorporation of a fucosyl group in Nod factors. A NAD(P)-binding site is present in the *NolK* amino acid sequence and the gene is homologous to *Escherichia coli* genes, presumably involved in GDP-fucose synthesis. Protein extracts of *A. caulinodans*, overexpressing *nolK*, have an enzyme activity that synthesizes GDP-fucose from GDP-mannose. *nolK* most probably encodes a 4-reductase performing the last step in GDP-fucose synthesis. Wild-type *A. caulinodans* produces a population of fucosylated and non-fucosylated molecules but the *nolK*-overexpressing strain produces only fucosylated Nod factors. Thus, the production of activated fucosyl donors is a rate-limiting step in Nod factor fucosylation.

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Terms	Documents
L4 same GDP-4-keto-6-deoxy-D-mannose	1

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<u>L6</u>	L4 same GDP-4-keto-6-deoxy-D-mannose	1	<u>L6</u>
<u>L5</u>	L4 same D-mannose	1	<u>L5</u>
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<u>L3</u>	epimerase or reductase	23381	<u>L3</u>
<u>L2</u>	GDP-4-keto-6-deoxy-D-mannose 3,5-epimerase-4-reductase	0	<u>L2</u>
<u>L1</u>	epimerase-4-reductase	1	<u>L1</u>

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L6: Entry 1 of 1

File: PGPB

Jan 31, 2002

DOCUMENT- IDENTIFIER: US 20020012979 A1  
TITLE: VITAMIN C PRODUCTION IN MICROORGANISMS AND PLANTS

Detail Description Paragraph (43) :

[0074] The ability to substitute GDP-4-keto-6-D-mannose epimerase/reductase for GDP-D-mannose:GDP-L-galactose epimerase to enhance L-ascorbic acid biosynthesis in plants or microorganisms depends on the ability of GDP-4-keto-6-deoxy-D-mannose epimerase/reductase to act directly on GDP-D-mannose to form GDP-L-galactose. Evidence supporting this possibility already exists. Arabidopsis thaliana murl mutants are defective in GDP-D-mannose-4,6-dehydratase activity (Bonin, et al., 1997, Proc. Natl. Acad. Sci. 94:2085-2090). These mutants are thus blocked in GDP-L-fucose biosynthesis, and consequently have less than 2% of the normal amounts of L-fucose in the primary cell walls of aerial portions of the plant (Zabrackis, et al., 1996, Science 272:1808-1810). The murl mutants are more brittle than wild-type plants, are slightly dwarfed and have an apparently normal life cycle (Zabrackis, et al., 272:1808-1810). When murl mutants are grown in the presence of exogenous L-fucose, the L-fucose content in the plant is restored to the wild-type state (Bonin, et al., 1997, Proc. Natl. Acad. Sci. 94:2085-2090). It was discovered (Zabrackis, et al., 1996, Science 272:1808-1810) that murl mutants contain, in the hemicellulose xyloglucan component of the primary cell wall, L-galactose in place of the normal L-fucose. L-galactose is not normally found in the xyloglucan component, but in murl mutants L-galactose partly replaces the terminal L-fucosyl residue. Bonin, et al. (1997, Proc. Natl. Acad. Sci. 94:2085-2090) hypothesized that in the absence of a functional GDP-D-mannose-4,6-dehydratase in the murl mutants, the GDP-4-keto-6-deoxy-D-mannose epimerase/reductase normally involved in L-fucose synthesis may be able to use GDP-D-mannose directly, forming GDP-L-galactose. Another possibility, however, is that the enzymes involved in L-ascorbic acid biosynthesis in A. thaliana are responsible for forming GDP-L-galactose in the murl mutant. If this were true, it would suggest that in the wild-type plant, some mechanism exists that prevents GDP-L-galactose formed in the L-ascorbic acid pathway from entering cell wall biosynthesis and substituting for (competing with) GDP-L-fucose for incorporation into the xyloglucan component (since L-galactose is not present in the primary cell wall of the wild-type plant).

Detail Description Paragraph (69) :

[0100] According to the present invention, a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase can be a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase from any organism, including Arabidopsis thaliana, Escherichia coli, and human. A nucleic acid sequence encoding a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase from Arabidopsis thaliana is represented herein by SEQ ID NO:1. SEQ ID NO:1 encodes a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase having an amino acid sequence represented herein as SEQ ID NO:2. A nucleic acid sequence encoding a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase from Escherichia coli is represented herein by SEQ ID NO:3. SEQ ID NO:3 encodes a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase having an amino acid sequence represented herein as SEQ ID NO:4. A nucleic acid sequence encoding a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase from homo sapiens is represented herein by SEQ ID NO:5. SEQ ID NO:5 encodes a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase having an amino acid sequence represented herein as SEQ ID NO:6.